

## The Brain-Derived Neurotrophic Factor Gene Confers Susceptibility to Bipolar Disorder: Evidence from a Family-Based Association Study

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Bipolar disorder (BP) is a severe psychiatric disease, with a strong genetic component, that affects 1% of the population worldwide and is characterized by recurrent episodes of mania and depression. Brain-derived neurotrophic factor (BDNF) has been implicated in the pathogenesis of mood disorders, and the aim of the present study was to test for the presence of linkage disequilibrium between two polymorphisms in the BDNF gene and BP in 283 nuclear families. Family-based association test (FBAT) results for the dinucleotide repeat (GT)<sub>N</sub> polymorphism at position –1040 bp showed that allele A3 was preferentially transmitted to the affected individuals ( $Z = 2.035$  and  $P = .042$ ). FBAT results for the val66met SNP showed a significant association for allele G ( $Z = 3.415$  and  $P = .00064$ ). Transmission/disequilibrium test (TDT) haplotype analysis showed a significant result for the 3-G allele combination ( $P = .000394$ ), suggesting that a DNA variant in the vicinity of the BDNF locus confers susceptibility to BP. Given that there is no direct evidence that either of the polymorphisms we examined alters function, it is unlikely that the actual risk-conferring allele is from these two sites. Rather, the causative site is likely nearby and in linkage disequilibrium with the 3-G haplotype that we have identified.

Family, adoption, and twin studies (Craddock and Jones 1999) have shown that bipolar disorder (MAFD1 [MIM 125480]) has a strong genetic component, and a non-Mendelian mode of inheritance, with more than one gene involved (McGuffin and Katz 1989; Gershon 1990). Animal studies have shown that brain-derived neurotrophic factor (BDNF) is implicated in adapting to stress exposure and in antidepressant response. Depressive states in animal models show a short- and long-term decrement in levels of BDNF in the hippocampus (Nibuya et al. 1995; Smith et al. 1995).

Recent reports indicate that antidepressant treatments, including electroconvulsive therapy, induce the expression of brain neurotrophins (Duman et al. 1998), suggesting that neurotrophin production in the brain in depressed patients may be deficient. Further evidence for the involvement of neurotrophins, and particularly for BDNF in depression, comes from studies in rats. BDNF was reported to promote the function and growth of serotonin (5-HT) neurons in the brain (Mamounas et al. 1999), and infusion of BDNF in the adult rat brain induced sprouting of 5-HT nerve terminals. (Siuciak et al. 1994, 1996). This

is of particular relevance, because, in major mood disorders, there is a decrease in brain 5-HT turnover in tissue and ventricular fluids. BDNF, being lipophobic and a relatively large protein, does not cross the blood-brain barrier. Therefore, 5-HT receptors, phosphodiesterase inhibition, and  $\beta$ -adrenoceptors appear to be implicated in the neuronal production of BDNF in some brain areas (Nibuya et al. 1995; Duman et al. 1997). Given that the principal treatment of depressive states in mood disorders consists of pharmacotherapy with selective serotonin reuptake inhibitors (SSRIs) and that BDNF plays a significant role in serotonin system development, the BDNF gene becomes an important candidate in mood disorders.

The BDNF gene (BDNF [MIM 113505]) was first reported, by Mainsonpierre et al. (1991) and Ozcelik et al. (1991), to be localized on the short arm of chromosome 11 (11p13) and was later mapped, by Hanson et al. (1992), at the boundary of 11p13 and 11p14. Linkage studies of the 11p region in BP have yielded mostly negative results, with notable exceptions that include a nonparametric LOD score of 1.89 in the 11p13–15 region (Detera-Wadleigh et al. 1999) and suggestive LOD scores (as high as 1.95) in the same region for BP in Costa Rican families (McInnes et al. 1996).

A valuable approach in the study of a disease with a complex mode of inheritance is the use of linkage disequilibrium (LD) analysis (Risch and Merikangas 1996), in which a particular locus is tested in parent-proband triads to detect association between the locus and the disease in the presence of linkage (Knapp 1999). Two

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**Table 1****Results of FBAT for BDNF Polymorphisms**

Locus and Allele	Frequency	No. of Families <sup>a</sup>	Statistic <sup>b</sup>	Expected Statistic <sup>c</sup>	Z	P <sup>d</sup>
(GT)n:						
1	.184	83	44.000	49.000	-.953	.340
2	.030	13	5.000	7.000	-1.000	.317
3	.700	93	135.000	123.750	2.035	.042
4	.065	40	17.000	20.250	-1.038	.299
5	.009	5	...	...	...	...
7	.003	3	...	...	...	...
8	.004	0	...	...	...	...
9	.004	3	...	...	...	...
10	.002	1	...	...	...	...
val66met:						
A	.231	115	51.000	73.000	-3.415	.00064
G	.769	115	185.000	163.00	3.415	.00064

<sup>a</sup> Number of informative families (i.e., families with at least one heterozygous parent).<sup>b</sup> Test statistic from FBAT for the observed number of transmitted alleles.<sup>c</sup> Expected value of S under the null hypothesis (i.e., no linkage or association).<sup>d</sup> One-tailed.

hundred eighty-three probands (119 men and 164 women) with a primary diagnosis of bipolar I ( $N = 182$ ), bipolar II ( $N = 100$ ), or schizoaffective disorder, manic type ( $N = 11$ ), mean age 34.2 years (SD 10.00), and mean age at onset of the illness 19.69 years (SD 7.34), were recruited, with their living parents, from hospital clinics and through newspaper advertisements in Toronto and across central Canada. Two hundred sixty-nine probands (95.0%) were of European origin, seven (2.5%) were Asian, four (1.4%) were Native American (aboriginal), and three (1.1%) were African American. The study was approved by the Research Ethics Board of the Centre for Addiction and Mental Health in Toronto. From all patients and their parents, written informed consent to participate in the study was obtained.

Blood (20 ml) was drawn from each subject, and DNA was extracted by use of the high-salt method (Lahiri and Nurnberger 1991). Subjects were genotyped for the BDNF dinucleotide-repeat polymorphism located 1,040 bp upstream from the transcription start site of the 1.6-kb BDNF mRNA (Proschel et al. 1992), and for the val66met SNP that determines a valine-to-methionine substitution at position 66 in the coding region. PCR was performed on 150 ng of template DNA to amplify a fragment containing the dinucleotide-repeat polymorphism in the putative promoter region of the BDNF gene. PCR products were subjected to electrophoresis on a 6% denaturing polyacrylamide gel for 2 h, after which the DNA was transferred to Whatman paper and exposed to x-ray film for 30 min. DNA bands were assigned allele numbers according to their size (allele 1 = 174 bp; allele 2 = 172 bp; allele 3 = 170 bp; allele 4 = 168; and allele 5 = 166).

The SNP for the G→A (valine→methionine) variation

at position 758 of the BDNF coding sequence was selected from the National Center for Biotechnology Information SNP database (reference number rs6265). A 113-bp segment was amplified by PCR, using the following primers: 5'-GAGGCTTGACATCATTGGCT-3' and 5'-CGTGTACAAGTCTGCGTCCT-3'. Target sequences were amplified in a 25- $\mu$ l reaction solution containing 125 ng genomic DNA; 1 U *Taq* polymerase (Sigma-Aldrich); 20 mM Tris-HCl (pH 8.4); 50 mM KCl; 1.5 mM  $MgCl_2$ ; 200  $\mu$ M each of dATP, dCTP, dGTP, and dTTP; and 10 pmol of each primer. After an initial denaturation of the DNA templates for 5 min at 95°C, 30 cycles were performed, each consisting of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. After the last cycle, samples were incubated at 72°C for 5 min. Samples were then digested overnight with 3 U of Eco721 (MBI Fermentas). The fragments were separated on a 3.5% agarose gel at 100 V, and fragments were visualized with ethidium bromide. The uncut product size was 113 bp (allele A), and allele G comprised the cut bands of 78 and 35 bp.

We tested for presence of LD between both BDNF polymorphisms and BP, using the family-based association test (FBAT), which allows for inclusion of both triads and extended families in the analysis. Transmission/disequilibrium test (TDT) for marker haplotypes (GENEHUNTER, version 2.1) was used to test transmission disequilibrium between haplotypes of the two BDNF polymorphisms and BP. In our sample, we found four common alleles of the dinucleotide-repeat polymorphism of the BDNF gene ( $A1 = 18.5\%$ ,  $A2 = 3\%$ ,  $A3 = 70.0\%$ , and  $A4 = 6\%$ ), with a heterozygosity of 47%. The total sample was in Hardy-Weinberg equilibrium when the rare genotypes were removed. The FBAT results (table 1) showed an excess transmission of allele

**Table 2****Results of Haplotype Analysis Using TDT**

Haplotype	Translated	Untranslated	$\chi^2$	<i>P</i>
1 A	20	39	6.12	.013376
1 G	8	8	.00	1.000000
2 A	0	1	1.00	.317311
2 G	6	8	.29	.592980
3 A	8	18	3.85	.049860
3 G	73	36	12.56	.000394
4 A	0	4	4.00	.045500
4 G	12	14	.15	.694887
5 G	2	2	.00	1.000000
7 G	1	1	.00	1.000000
10 A	1	0	1.00	.317311

A3 from parents to the offspring ( $P = .042$ ). We also tested for parent sex-specific transmission, but no parent-of-origin effect was detected in the transmission of *BDNF* alleles. Frequencies for the alleles of the val66met polymorphism were A = 23.1% and G = 76.9%. FBAT analysis of this SNP showed  $Z = 3.415$  ( $P = .00064$ ) (table 1). Haplotype analysis, using TDT, resulted in  $\chi^2 = 12.56$  ( $P = .00039$ ) for the 3-G combination (table 2). An examination of the sample for association between the presence/absence of risk haplotype and age at onset, presence of psychotic symptoms, and rapid cycling did not yield significant results. The 1-A haplotype had significantly decreased transmission to probands with BP, suggesting the possibility that it is protective against the disorder. The degree of LD between the two markers, as determined by the two-locus LD program (Klitz et al. 1995), generated a  $D'$  value of 0.695 for the 3-G haplotype and of 0.773 for A1, with a global disequilibrium  $\chi^2 = 822.25$  (3 df;  $P < 1 \times 10^{-7}$ ). The size of our sample is large enough to guarantee reasonable power for the LD analysis performed (McGinnis 2000).

The present study is the largest to date of *BDNF* and mood disorder. The results strongly suggest LD between both the GT repeat and val66met markers and BP. The presence of LD between *BDNF* and BP implies, in turn, that this locus may be involved in the pathogenesis of the disease. Further evidence for a role of *BDNF* in bipolar disorder has been reported by Sklar et al. (2002).

Brain imaging studies of patients with BP and unipolar depression have demonstrated morphometric changes that suggest cortical atrophy and/or cell death in these patients (Elkis et al. 1995; Sheline et al. 1996, 1999; Drevets et al. 1997, 1999; Soares et al. 1997; Steffens et al. 1998). *BDNF* is a neurotrophin found primarily in the neocortex, hippocampus, and amygdala (Buchman and Davies 1993; Ip et al. 1993; Korsching 1993; Duman 1999). Most of the work, to date, that supports the role of *BDNF* in depression has been derived from studies in animals. *BDNF* may be implicated in the etiology of BP in humans by affecting the mechanisms in-

involved in cell formation, cell death, and/or neuroplasticity. *BDNF* is only one molecule among many others, such as glutamate, that might be implicated in neuronal survival (Moghaddam et al. 1994).

Because BP overlaps extensively with other mood disorders, including unipolar depression, our significant findings with *BDNF* may be applicable to depression in general (King et al. 2001). Given the overlap of bipolar disorder and schizophrenia in some genetic linkage studies, the question arises as to whether *BDNF* also plays a role in schizophrenia. The *BDNF* (GT)<sub>n</sub> dinucleotide repeat has been tested for association with schizophrenia in six independent studies (Sasaki et al. 1997; Hawi et al. 1998; Wassink et al. 1999; Krebs et al. 2000; Virgos et al. 2001; Muglia et al. [in press]). Three of these studies (Sasaki et al. 1997; Krebs et al. 2000; Virgos et al. 2001) used a case-control association strategy, two studies (Wassink et al. 1999; Muglia et al. [in press]) used a family-based approach, and a fifth study used both the family and the case-control approach (Hawi et al. 1998). Among these studies, none detected association between the *BDNF* dinucleotide repeat and schizophrenia, except the work reported in the article by Muglia et al. (in press). It is possible that our schizophrenia sample, compared with others published, gave different results because we included subjects with schizoaffective disorder, depressed subtype, and because we focused on a primarily Italian sample. In another phenotypic variation, Krebs and colleagues (2000) reported the presence of an association between a group of *BDNF*-dinucleotide long alleles (172–176 bp) and patients with late-onset schizophrenia that responded to neuroleptics. Overall, the data to date do not suggest a strong overlap in the effect of *BDNF* between bipolar disorder and schizophrenia. Depression, in turn, is the most common of all the psychiatric disorders, and it represents one of the leading health problems worldwide (Murray and Lopez 1997), along with cardiovascular and infectious diseases. Thus, if the positive associations of *BDNF* in mood disorders can be replicated, the relevance to world health may be highly significant.

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## Electronic-Database Information

URLs for data presented herein are as follows:

National Center for Biotechnology Information <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp>

Online Mendelian Inheritance in Man (OMIM) <http://www.ncbi.nlm.nih.gov/Omim/>

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